DIETARY BIOACTIVE COMPOUNDS AS HISTONE DEACETYLASE INHIBITOR FOR CANCER PREVENTION

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Submitted By

DEBADUTTA BHOI

Roll No – 410LS2043

Under the guidance of

Dr. SAMIR KUMAR PATRA

Associate Professor and Head

DEPARTMENT OF LIFE SCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA, ODISHA

DEPARTMENT OF LIFESCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA-769008

.....

Dr. SAMIR KUMAR PATRA

Associate Professor and Head.

Ref. No…….....

Date: .....

CERTIFICATE

This is to certify that the thesis entitled "**Dietary bioactive compounds as histone deacetylase inhibitor for cancer prevention**" which is being submitted by Mr. **Debadutta Bhoi**, Roll No. **410LS2043**, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bona fide research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Dr. SAMIR K. PATRA

Associate Professor and Head,

Department of Life Science

National Institute of Technology

Rourkela – 769008

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Finally all credit goes to my parents and my friends for their continued support.And to all mighty, who made all things possible……..

DEBADUTTA BHOI

DECLARATION

I, Debasutta Bhoi, hereby declare that this project report entitled "**Dietary bioactive compounds as histone deacetylase inhibitor for cancer prevention**" is the original work carried out by me under the supervision of Dr. Samir K. Patra, Department of Life Science, National Institute of Technology Rourkela (NITR), Rourkela. To the best of my knowledge and belief the present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree or diploma.

Debadutta Bhoi

Place:

Date:

CONTENTS

Abstract

Cancer is major disease causing worldwide human death which is related to different epigenetic dysregulation. One of the major dysregulation is overexpression of histone deacetylase (HDAC) including, HDAC1, HDAC4, HDAC6 and HDAC7. HDAC upregulation leads to histone deacetylation at the promoter region of tumor suppressor genes and promote histone 3 lysine 9 methylation helping in heterochromatinization, leading to abnormal changes in chromatin pattern followed by tumorigenesis. In this project novel HDAC inhibitor finding will focus a new light on cancer therapy. Our normal diet of fruits and vegetables, contain different bioactive compounds like sulforephine, allyl disulfite and butyrate, which have anti-cancerous property. Insilco study illustrate that these bioactive compound inhibit HDAC 4, 7 and 6 by binding with the active site of HDAC enzyme. This HDAC-bioactive compound complex become inactive and fails to remove acetyl group from histone tail which helps in cancer inhibition. Further experimental research will confirm the evidence of these novel inhibitors.

1. INTRODUCTION

Epigenetics is the change in the expression of the genes without change in the sequence of the DNA. The term 'epigenetic' was coined by the developmental biologist, Conrad Hal Waddington, in 1942. Cancer is a multi-step process derived from combinational crosstalk between genetic alterations and epigenetic influences through various environmental factors (Ducasse and Brown 2006; Esteller 2008; Ellis *et al.,* 2009). Environmental exposure to nutritional, dietary, chemical and physical factors can alter gene expression and modify individual genetic susceptibility through changes in the epigenome (Issa 2008; Suter and Aagaard-Tillery 2009; Herceg 2007).

Epigenetic mechanisms controlling the gene transcription are mainly involved in proliferation, differentiation and survival of the cell and are casually linked with malignant development. Epigenetic processes like chromatin modifications such as DNA methylation and histone acetylation are common targets studied in cancer epigenomics (Herceg 2007; Esteller 2007). In cancers it was observed that, half of all tumor suppressor genes are suppressed more due to epigenetic, than by genetic, mechanisms (Issa 2008). One hallmark of human cancers is the loss of monoacetylation and trimethylation of histone H4 (Fraga *et al.,* 2005)

Inhibition of HDAC disturbs the cell cycle in G2, allowing cells to prematurely enter the M phase and also interfere directly with the mitotic spindle checkpoint. Amazing thing is that, HDAC inhibitors appear to activate cell cycle arrest and apoptosis more effectively in cancer cells than in non-transformed cells, although the mechanisms are not elucidated.

Bioactive compounds can destroy the cancer cells by targeting the abnormal pattern of histone modification, i.e. HDAC inhibitor Trichostatin A (TSA) and its structural analogs, which are potent agents used for cancer therapy. Dietary compounds like sulforaphane (SFN), diallyl disulfide (DADS), and butyrate act as weak ligands for HDAC and exhibit HDAC inhibitory activity (Marks 2007).

Growing evidence suggests that bioactive dietary components impact different epigenetic processes like activation of cell survival proteins, reactivation of tumor suppressor genes, and

induction of cellular apoptosis in different types of cancer (Landis-Piwowar *et al.,* 2008; Li *et al.,* 2010; Paluszczak *et al.,* 2010; Majid *et al.,* 2008).

There are various types of epigenetic processes are DNA methylation, histone modification, RNA interference, all these play central role in gene expression and maintenance of genome.

1.2 Histone Modification

Histones are highly alkaline proteins found in nuclei of the eukaryotic cells. This group of protein bound to the DNA and forms a structural unit called nucleosome.

They are the chief protein component of chromatin, around which DNA winds, and mediate gene regulation. Histone proteins help in packing of DNA. The unwound DNA in chromosomes would be very long without histones (a length to width ratio of more than 10 million to one in human DNA)

Many histone modifications play important roles in epigenetic alterations. Acetylation and methylation are the two most important histone modifications that play crucial role in cancer. (Davis and Ross 2007; Fraga *et al.,* 2005; Seligson *et al.,*2005). These two histone modification bring on chromatin changes that allow access to the various transcriptional activators and/or repressors at gene promoters. Therefore these two histone modification play a crucial role in gene regulation and tumorigenesis (Ganesan *et al.,* 2009; Dalvai and Bystricky 2010; Sharma *et al.,* 2010). Different types of reversible covalent posttranslational modifications including occur in the histone protein including, lysine and arginine methylation, lysine acetylation, lysine ubiquitination and sumoylation and serine and threonine phosphorylation, and these modifications take place mainly among the histone amino-terminal tails projecting from the surface of the nucleosome as well as on the globular core area and regulate fundamental cellular processes like transcription, replication, and repair (Kouzarides 2007). Particular histone modifications seems to act as programmed “codes” which can be recognize by specific proteins to bring about specific downstream events such as transcriptional activation or repression. Here our main focus is on the Histone deacetylation which occurs in the lysine by removal of acetyl group.

Histone modifications are catalyzed by many enzymes such as histone acetyltransferases (HATs), histone deacetylases (HDACs). HATs and HDACs add and remove acetyl group to the lysine residues present in histones, respectively (Choudhuri *et al.,* 2010). Histone acetylation increases chromatin accessibility by neutralizing the DNA–histone interactions which leads in a relaxed, open chromatin formation that permits the transcriptional activators to gain access to their cognate recognition components and initiate or enhance transcription (Görisch *et al.,* 2005; Lafon-Hughes *et al.,* 2008). Histone hypoacetylation at promoter region, caused by either lack of HAT activity or increased HDAC activity, consequence in silencing of the tumor suppressor gene like p21WAF1/CIP1 in tumorigenesis (Majid *et al.,* 2008; Kikuno *et al.,* 2008). But interestingly histone hyperacetylation at certain promoters regions by either higher HAT activity or reduced HDAC activity, results in normal repressed gene activation (Acharya *et al.,* 2005; Kim *et al.,* 2003).

1.3 Histone deacetylases

Histone deacetylases (HDAC) are the class of enzymes which remove acetyl groups from an ε-N-acetyl lysine amino acid on a histone protein leading to decrease in the space between the nucleosome and the DNA that is wrapped around it. Tighter wrapping of the DNA around the nucleosome block interaction between transcription factors and DNA lead to transcriptional repression (Strahl and Allis 2000). The catalytic domain of HDAC is made by a stretch of about 390 amino acids consisting of a set of conserved amino acids (Finnin *et al.,* 1999). The active site comprises of a lightly curved tubular pocket with a wider bottom. Removal of an acetyl group takes place through a charge relay system comprising of two neighboring histidine residues, two aspartic residues (located more or less 30 amino acids from the histidines and separated by about 6 amino acids) and one tyrosine residue (located approximately 123 amino acids downstream from the aspartic residues) (Finnin *et al.,* 1999). An essential constituent of this charge-relay system is the presence of a Zn^{++} ion as shown in figure 1 and 2. This atom is tied to the zinc binding site on the bottom of the pocket. HDAC inhibitors work by moving the zinc ion from its original position causing the charge-relay system dysfunctional. TSA, with its five-carbon atom linker to the phenyl group and hydroxamic acid group, has the optimal conformation to fit into the active site. (Finnin *et al.,* 1999) TSA is one of the most effective

reversible HDAC inhibitors presently known, with IC_{50} and SAHA which works in very low quantity like few nano molar range (Yoshida *et al.,* 1990).

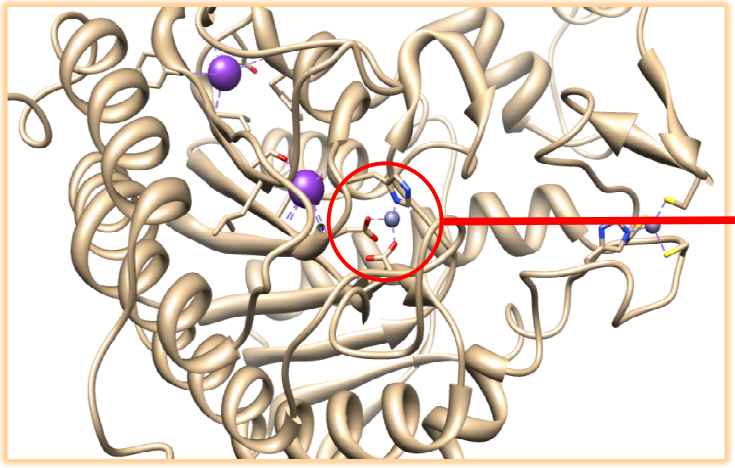


Fig1: Structure of HDAC 7
(active site of enzyme under red circle)

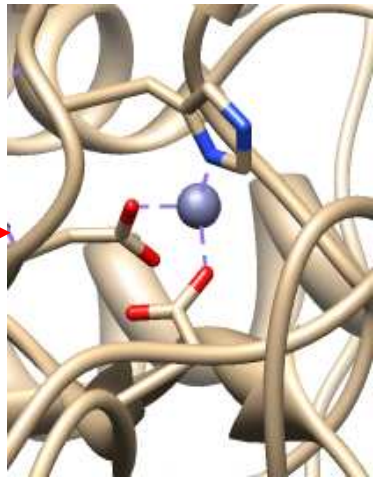


Fig2: Active site of HDAC7

1.4 HDAC family

Histone deacetylases (HDACs) catalyse the removal of acetyl groups from lysine residues from amino termini of histone causing chromatin condensation and transcriptional repression (Roth at al. 2001; Thiagalingam *et al.,* 2003) Till now eighteen HDACs have been identified in humans. According to their homology to yeast, subcellular localization and enzymatic activities HDACs are subdivided into four classes, (Thiagalingam *et al.,* 2003). The classes I HDACs (1, 2, 3 and 8) are homologous to the yeast RPD3 protein, generally present in the nucleus and show ubiquitous expression in various human cell lines and tissues. Class II HDACs HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10 share homologies with the yeast Hda1 protein and shuttle between the nucleus and the cytoplasm. The classes IIb HDACs are HDAC6 and HDAC10 and they are generally found in the cytoplasm and contain two deacetylase domains. The class III HDACs (SIRT1, 2, 3, 4, 5, 6 and 7) are homologues of the yeast protein Sir2 and require NAD+ for their activity; they regulate gene expression according to changes in the cellular redox status. At last the class IV HDACs, HDAC11 is the only enzyme reported under these class. It shows sequence similarity with the catalytic domain of both class I and II

HDACs enzymes but does not have strong enough identity to be placed in either class (Catley *et al.*, 2003).

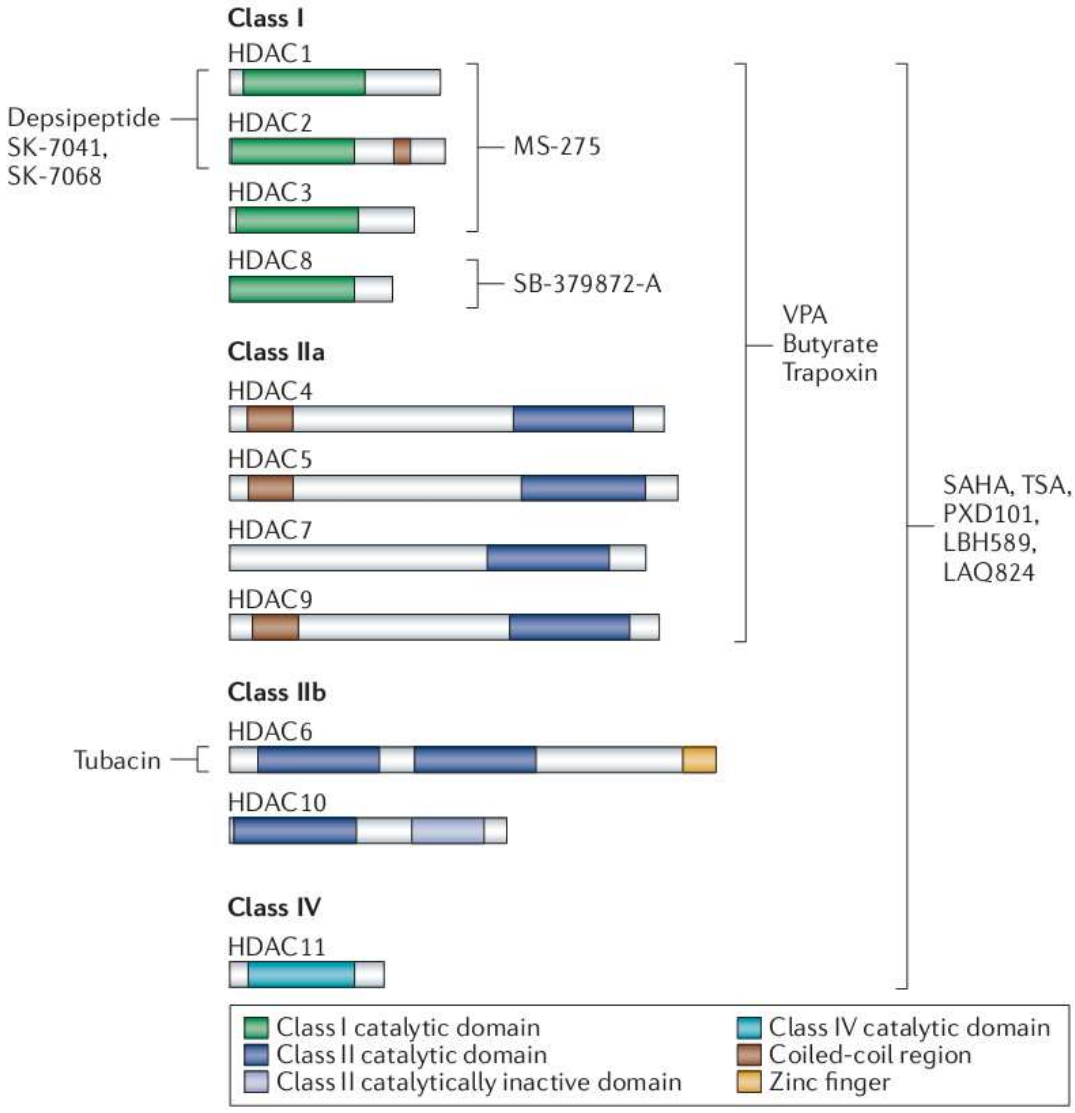


Fig3: Different class of HDACs (Bolden *et al.*, 2006)

1.5 HDAC inhibitors

Histone deacetylase inhibitors (HDACi) are a class of compounds that intervene with the function of histone deacetylase by displacing the Zn^{++}. Without Zn^{++} HDAC cannot recognize the acetyle group and unable to remove it from histone leading to euchromatin formation. There are a lot of inhibitors but most potent observed so far is Trychostatin (TSA) which is a fermentation product of *Streptomyces*. A large number of HDACi have been purified from natural sources or synthetically prepared.

1.5.1 HDAC inhibitor in the diet

For more than a decade, there has been substantial concern in the use of naturally occurring drugs for the prevention of diseases, including cancers. Vegetables, fruits, Beverages, and other elements of the human diet normally contain polyphenols which have been demonstrated in many investigations to have chemopreventive and anti-cancer property. (Aggarwal and Shishodia 2006; Meeran and Katiyar 2008; Yang *et al.,* 2001). Various nutrients, specifically dietary drugs, can play a major role in the regulation of both normal and pathological process. Better understanding of the regulative role of these nutrients on several molecular targets may help in the prevention and treatment of different type of cancers. Several dietary compounds or nutrients govern various molecular targets in different type of cancers. Sulforaphane from cruciferous vegetables, genistein from soybean, tea polyphenol-catechins from green tea, curcumin from turmeric, diallyl disulfide from garlic, resveratrol from grapes, and other bioactive compounds like cyanidins from grapes apigenin from parsley, isothiocyanate from cruciferous vegetables, baicalein from Indian trumpet and rosmarinic acid from rosemary (Meeran *et al.,* 2010). These bioactive compounds target various epigenetic targets like HDAC, DNA methyl Transferase etc.

We mainly focused on the HDAC inhibitors such as sulforaphane, allyl disulfide and butyrate. So we performed molecular docking of different HDACs (4, 6, 7) with sulforaphane, diallyl dsulfide and butyrate. Comparative study using bioinformatics tool shows that sulforaphane show more efficiently inhibiting the HDACs in comparison to diallyl disulfide and butyrate. It can be an ultimate dietary drug for cancer prevention.

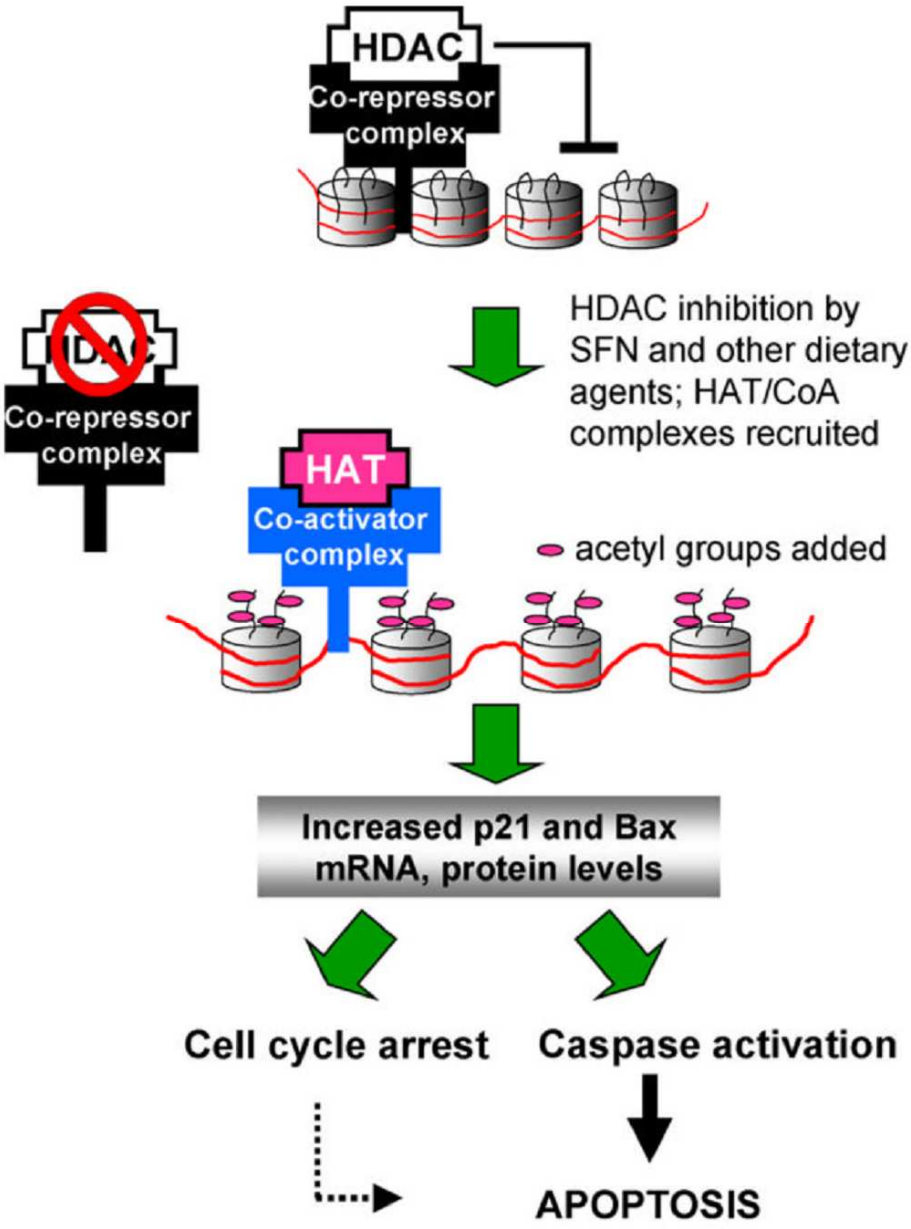


Fig4: Role of dietary histone deacetylase (HDAC) inhibitors in cancer cell (Meeran *et al.*, 2010 Semin Cancer Biol. 2007 October ; 17(5): 363–369. doi:10.1016/j.semcancer.2007.04.001.NIH-)

2. REVIEW OF LITERATURE

2.1 Role of HDAC in cancer

There are no decisive data regarding the pattern of HDAC expression in human cancer, but there are a number of studies showing altered expression of different type HDACs in different tumor samples.

Altered regulation of DNA methylation and post-translational histone modifications are characteristic hallmarks of human cancers. Fraga *et al.*, 2005 shown that, loss of acetylated Lys16 (K16-H4) and trimethylated Lys20 (K20-H4) of histone H4 is a common consequence in human cancer that is linked with the hypo-methylation of repetitive sequences in primary tumors and human cancer cell. Moreover, they shown the data from a mouse model of multistage skin cancer tumorigenesis, indicating that the global loss of monoacetylated and trimethylated forms of histone H4 is a crucial event in cancer development and these changes occur early in tumorigenesis

Yasui *et al.*, 2003 observed reduction in histone acetylation is not only involved in introduction of tumor genesis but also in tumor invasion and metastasis in gastro intestinal tumors. Santiago Ropero and Manel Esteller have reported an increase in expression of HDAC 1 in breast, gastric, colon and prostate cancer, over expression of HDAC 2 in cervical, gastric cancer and in colorectal carcinoma with loss of APC expression, high levels of HDAC3 in colon cancer and HDAC6 expression and breast cancer specimens.

2.2 Effect of histone deacetylase inhibitors in cancer cell

It have been reported that HDACi (HDAC inhibitors) disrupt the cell cycle in G2 and allow the cells to prematurely enter the M phase, as well as interrupting the mitotic spindle checkpoint. HDAC inhibitors appear to initiate cell cycle arrest and apoptosis more specifically in cancer cells than in normal cells, the mechanisms for this is still not elucidated. Recent studies have implicated the induction of pro-apoptotic genes like TRAIL (TNF-related apoptosis-inducing ligand), DR4 (Death Receptor 4) and DR5 (Death Receptor 5) occur only in transformed cells (Ungerstedt *et al.*, 2005; Nebbioso *et al.*, 2005). The fundamental epigenetic differences between normal and tumour cells could alter the transcriptional response to HDACi. It has been already

proved that global hypo-acetylation of histone H4 is a common hallmark of human tumours. Alterations in H4 acetylation occur early in the tumorigenic process (Fraga *et al.*, 2005). Reestablishment of a normal epigenetic condition by HDACi, or selective induction of tumour-suppressive genes like TRAIL by HDACi specifically in the context of a neoplastic epigenome', could be clinically beneficial (Bolden *et al.*, 2006).

Patra *et al.*,, 2001 to demonstrate that DNMT1 and HDAC1 levels are up-regulated in prostate cancer. They reported that HDACs activities were two- to threefold higher in prostate cancer cell lines compared to benign prostatic hyperplasia (BPH-1) cell line.

Activation of either an extrinsic or intrinsic pathway or both of these cell death pathways by all the HDAC inhibitor is observed in many cancer models (Rosato *et al.*, 2003). Xu *et al.*,, 2008 reported that when the ligands, like Fas or TRAIL bind to their death receptors they activate the death-receptor pathway. Without altering the levels of DR4 or DR5 in breast cancer cells MS275 (entinostat) and SAHA induce TRAIL expression, which was mediated through SP1 and cause increase adriamycin cytotoxicity in breast cancer cells All these consequences resulted in the recruitment of an adaptor protein, FADD, and the activation of caspase-8. Stress stimuli by chemotherapeutic agents disrupt the mitochondrial membrane, leading to the release of proteins, including cytochrome *c* and SMAC. Apoptosome formation and activation of caspase-9 is mediated by Cytochrome *c* release. Caspases-3, -6, and -7 then cleaved by caspase-8 and caspase-9, leading to apoptosis. Mitochondrial disfunction and apoptosis by XIAP down-regulation increase in ROS generation and JNK1 activation is cause by SAHA and MS275. (Xu *et al.*, 2008)

Fulada .S shown that, treatment of various cancer cell with HDAC inhibitor like FK228, TSA, LBH589 and SAHA show amplification of intrinsic as well as extrinsic apoptotic pathways due to decrease in the expression of Bcl-2, Bcl-xL and XIAP, and an increase in the expression of pro-apoptotic proteins, such as Bak and Bax, is cause enhance TRAIL-mediated cytotoxicity in various type of cancer cells

2.3 Dietary bioactive compound as HDAC inhibitor

Phytochemicals or dietary bioactive compounds are non-nutritive elements in the plant which possess significant antimutagenic and anticarcinogenic properties. There is a great structural

diversity in the phytochemicals structure and activity relationships to deduce their underlying molecular mechanisms. Elucidation of signal transduction pathways is a better approach to analyze their effects on cancer.

Young-Joon Surh, 2003 conduct studies on more than 250 people population, including case control and cohort studies and observed that, Those people who eat fewer than two servings are more prone to cancer, specially cancers in the digestive and respiratory tracts as compare to people who eat about five servings of fruit and vegetables a day (about half the risk of developing cancer)

The NCI has reported around 35 plant-based foods that have anticancer properties including cruciferous vegetables (cabbage, broccoli, cauliflower etc.), turmeric, garlic, soybeans, ginger, onion and tomatoes etc

2.3.1 Sulforaphane

Sulforaphane is an organ sufur compound, mostly present in cruciferous vegetable like broccoli, cabbage, cauliflowers etc and exhibits some excellent properties like antidiabetic, anticancer and antimicrobial properties in various experimental models.

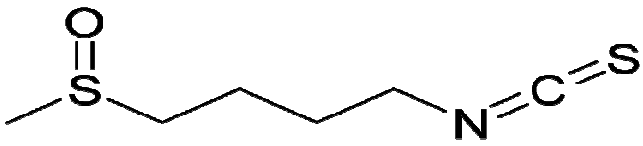


Fig 5: Chemical structure of sulforaphane (wikipwdia.org)

2.3.2 Allyl disulfide

Allyl Disulfide is an organosulfur compound produced in garlic. It is a yellowish color water insoluble liquid and has a strong garlic odor. It matches butyrate in having a spacer terminating with a carboxylic acid functional group.

Fig 6: Chemical Structure of Allyl Disulfide (wikipwdia.org)

2.3.3 Butyrate

Butyric acid is present in butter, Parmesan cheese, and vomit, and is a product of anaerobic fermentation in our body. It is a fatty acid present in the form of esters in plant oils and animal fats

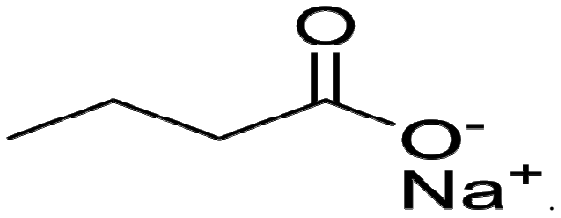


Fig7: Chemical structure of Butyrate (Wikipedia.org)

Butyrate comprises a short three-carbon spacer attached to a carboxylic acid group, which may enter into the active site of the enzyme and forms a bidentate ligand with the zinc atom.

3. Objective

- ✓ Study of interaction between bioactive compounds (sulforaphane, allyl disulfide, autyrate) as inhibitor and HDAC (4, 6, 7) using Bioinformatics Tools
- ✓ Comparative study among Sulforaphane, butyrate and allyl disulfide as HDAC inhibitor.

4. MATERIALS AND METHOD

The structural details of the following HDACs (4, 6, and 7) proteins present in *Homo sapiens* were obtained from structural database of "Protein Data Bank" (PDB) (http://www.rcsb.org/pdb). The structures are conventionally represented by specific 4 letter code of pdb id such as 2VQM, 3PHD, 3C0Y for respective proteins mentioned above.

4.1 Software Required

- ✓ Chimera
- ✓ Swiss Pdb Viewer
- ✓ Hex
- ✓ LIGPLOT^{+}
- ✓ Open Babel

4.2 Preparation HDAC enzymes of *Homo sapiens* for docking

Chimera

It is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, sequence alignments, docking results, trajectories, conformational ensembles and supra-molecular assemblies. High-quality images and animations can be generated. Chimera is developed by the Resource for Bio computing, Visualization, and Informatics, funded by the National Institutes of Health National Center for Research Resources and National Institute of General Medical Sciences

Protocol

The non-standard residues in the structural pdb files of HDAC proteins were edited using Chimera. The removal of the residues resulted in energetically unstable protein. The proteins were stabilized by the process of energy minimization using "Swiss PDB Viewer". Subsequently HDACs (4, 6 and 7) proteins were uploaded to Hex, and polar hydrogen was added to each of those HDAC. The charge on Zinc (Zn) ions of the proteins was converted from 0 to +2 by using python script. They were saved in pdb format.

4.3 Preparation of HDAC enzymes of *Homo sapiens* inhibitor files

The 3D structural details of ligands such as Sulforaphane, allyl disulfide and butyrate were downloaded from "Pub Chem" structure data base at NCBI site (ncbi.nlm.nih.gov) and the compound id were 5350, 16590, 104775 respectively. All the files were in SDF format. The SDF files format was converted to PDB format file using "Open Bable Software". The ligands in PDB format were loaded with Auto Dock. The torsion of the ligands was adjusted based on the total number of rotatable bonds. The ligands were saved in PDB format.

4.4 Docking

Hex v6.3

It was written by dave Ritchie. It is an interactive protein docking and molecular superposition program. *Hex* understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files.

Protocol

The pdb files of receptor HDAC proteins and ligands were uploaded on Hex working platform and then it followed docking. The binding energy details of proteins were saved in log file and structural complex as pdb files.

4.4 Analysis of Binding sites by LIGPLOT^{+}

LIGPLOT^{+}

It is graphical user interface version of LIGPLOT and DIMPLOT programs. The plots generated by these programs can be interactively edited on screen, superposed and printed.

Protocol

The interacting residues of protein and ligand were analyzed using LIGPLOT $^{+}$ software and analyzed for best ligand for the HDAC 4, 5, and 7.

5. Result and Discussion

5.1 Interaction of the inhibitor with HDACs

The docking result obtain by Hex was saved in PDB format to visualize in the LIGPLOT software to identify the interacting residues. The residues details are described in the table 1 below. It has been identified that sulphoraphane interact with the following residues (Glu 221, **Asp 178**, His 155, **Zn^{2+}**, Asp 132, Ala 332, and Ile 329) of HDAC 4 (Fig 8). Similarly on interaction with HDAC 6 following reduies Asp **71**, **Zn^{2+}**, Ala 46, Phe 45 were identified (Fig 9). But it has observed that sulforaphane interact with sites other than the active sites residues. HDAC 7 (Fig10) .Thus it can be inferred as HDAC 4 and 6 can be inhibited by sulphoraphane while it is unable to inhibit HDAC 7. In case of HDAC 4 and 6 sulforaphane is able to bind to Zn^{2+} ion and one of the amino acid residue of active site (Asp 178 and Asp71 respectively) (fig 8 nad 9). Due to this reason it may inhibit the enzyme by distressing the Zn^{2+} ion. Similarly the inhibitor "Allyl disulfide" interact with **Asp272, Zn2+,** Tyr319, Ile329, Glu 328, Asp326 and Val323 residues of HDAC 4 (fig 11) while Leu8 and Lue 71 residues in HDAC 6 (fig 12) and **Asp 285**, Arg79,Cys 397, Met 298 and Ile280 residues were identified in HDAC 7 (fig 13). Subsequently on analysis of interaction with inhibitor "Butyrate" following residues were identified to be Cys169, Phe168, **Asp178**, Pro165, Met 166 and **Zn^{2+}** in HDAC 4 (fig 14) and Ala 46 of HDAC 6 (fig15) and Asp191 and Pro 293 of HDAC 7 (Fig16). Butyrate binds to Zn^{2+} ion and active site residue (Asp 178) of HDAC4 but in case of HDAC 6 and HDAC 7 it unable to bind to the active site and Zn^{2+}. Allyl disulfide and butyrate both unable to bind to active site and Zn^{2+} thus cannot inhibit HDAC 6 and HDAC 7 .In all cases the interaction between ligand and Zn++ ion is electrostatic interaction where as the interaction between the active site amino acid residue and ligand is hydrophobic,van der waals and hydrogen bond.

Table 1: **Active Site residue of HDAC (4, 6, and 7) (Tambunan *et al.* 2011, (Suppl 13):S23,)**

Enzyme	Active site residues
HDAC 4	Ion Zn^{2+}, Asp193, His195, and Asp287
HDAC 6	Ion Zn^{2+}, Asp71, His73, and Asp164
HDAC 7	Ion Zn^{2+}, Asp191, His193, and Asp285

Table 1 : **Interactions of Sulforaphane, Allyl Disulfide and Butyrate with HDAC 4,6,7**

Ligands/Protein	HDAC4	HDAC6	HDAC7
Sulforaphane	Glu221, **Asp178**, His155, **Zn^{2+}**, Asp132, Ala332, Ile329	**Asp71**, **Zn^{2+}**, Ala 46, Phe45	Arg154**,** Gly 36, Leu19, Leu 105
Allyl disulfide	**Asp272, Zn2+,** Tyr319, Ile329, Glu 328, Asp326, Val323	Leu8, Lue 71	**Asp 285**, Arg79, Cys 397, Met 298, Ile280
Butyrate	Cys169, Phe168, **Asp178**, Pro165, Met 166, **Zn^{2+}**	Ala46	**Asp191**,Pro293

* Residues of active site written in bold letter.

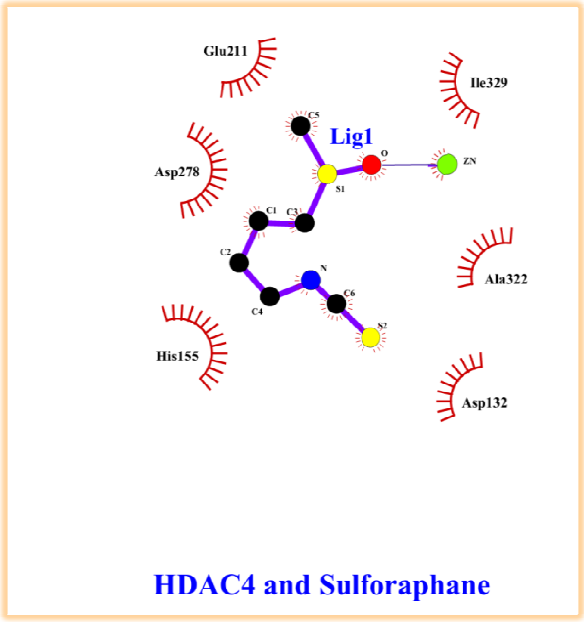


Fig 8: Interaction between HDAC4 and sulforaphane

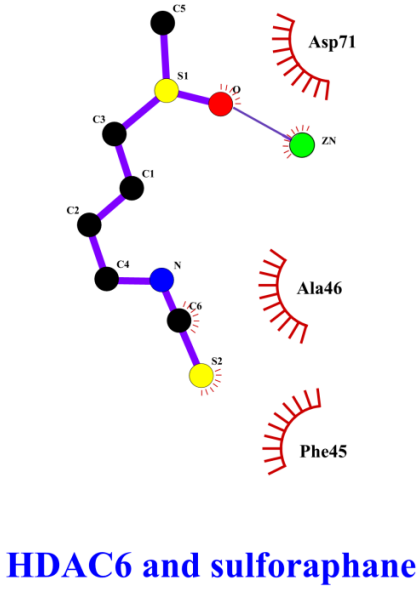


Fig 9 Interaction between HDAC6 and sulforaphane

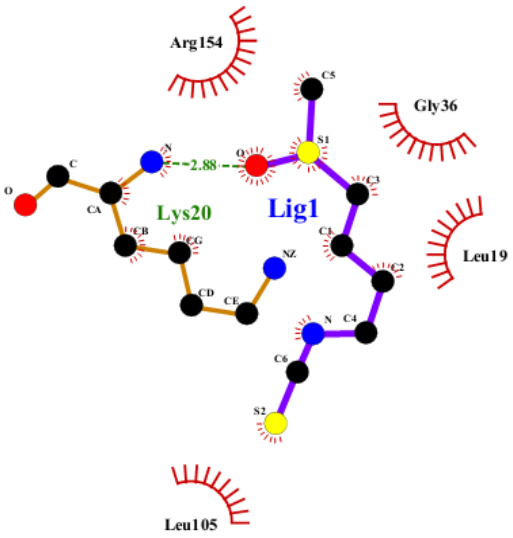


Fig 10: Interaction between HDAC7 and sulforaphane

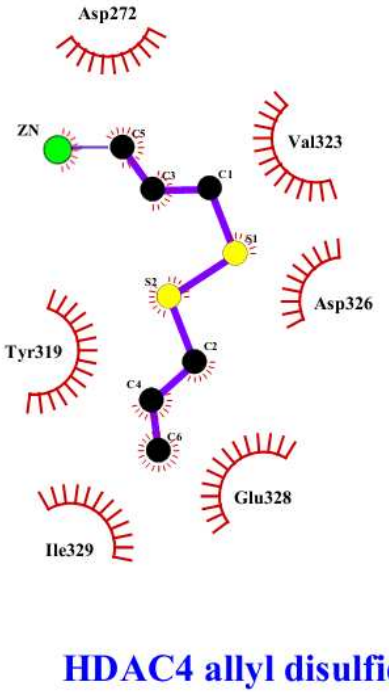


Fig 11: Interaction between HDAC4 and allyl disulfide

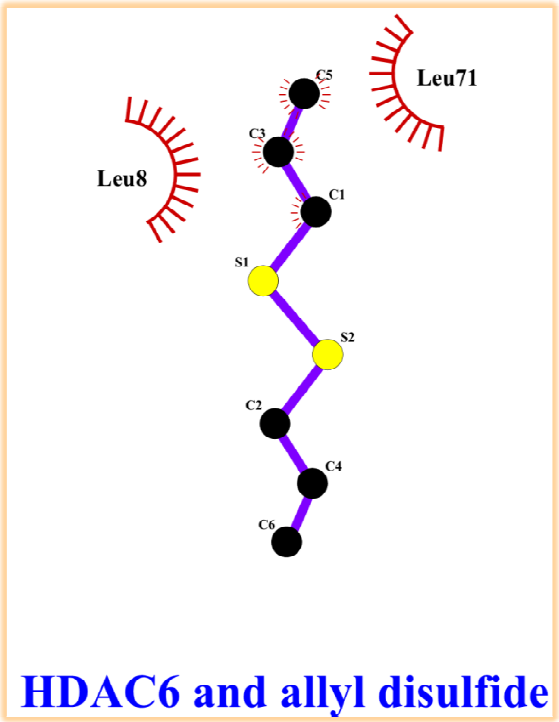
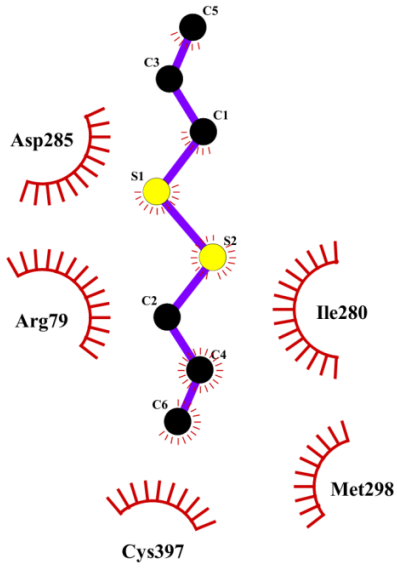


Fig 12: Interaction between HDAC 6 and allyl disulfide



HDAC7 and alllyl disulfide

Fig 13: Interaction between HDAC 7 and allyl disulfide

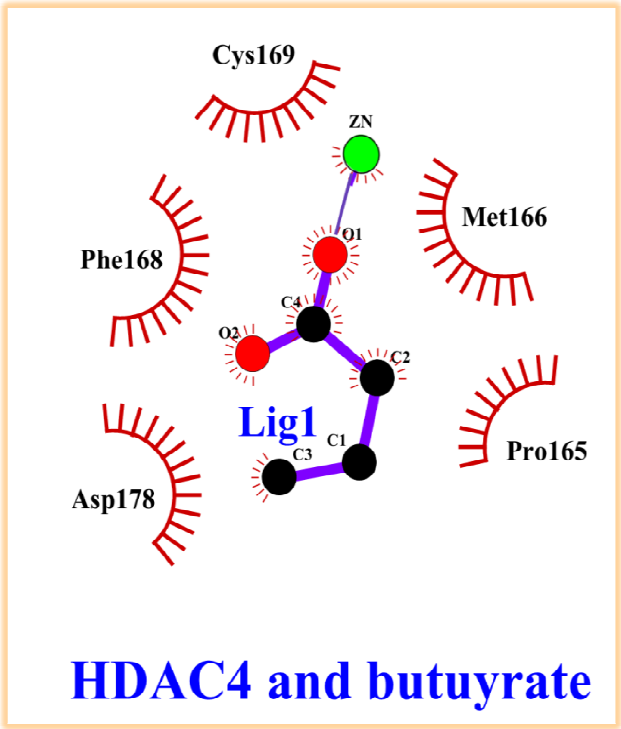


Fig 14: Interaction between HDAC4 and butyrate

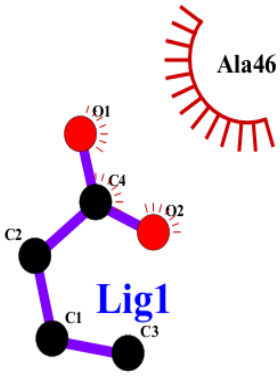


Fig 15: Interaction between HDAC 6 and butyrate

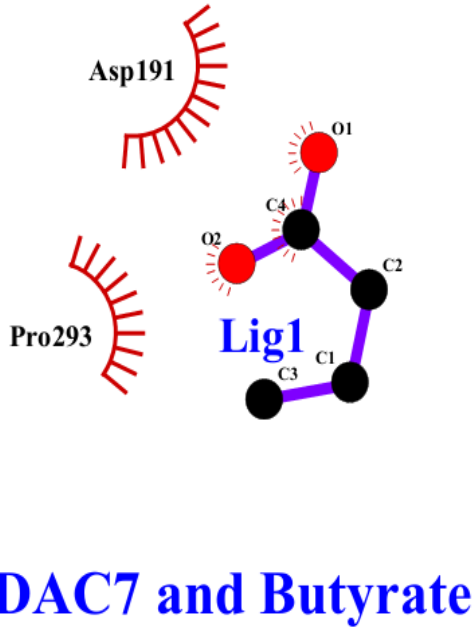


Fig 16: Interaction between HDAC 7 and butyrate

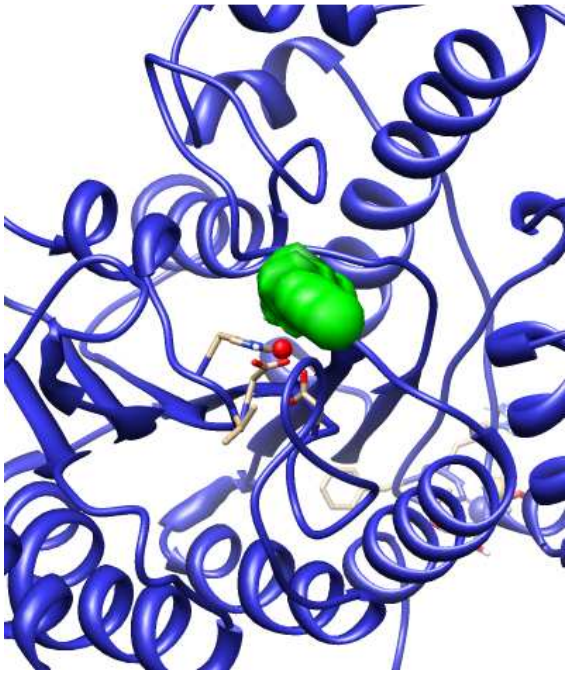


Fig17: HDAC4 and sulforaphane

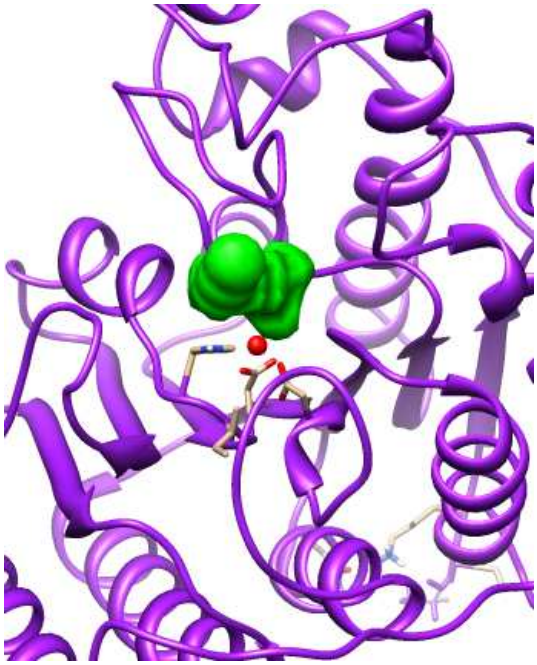


Fig18: HDAC6 and sulforaphane

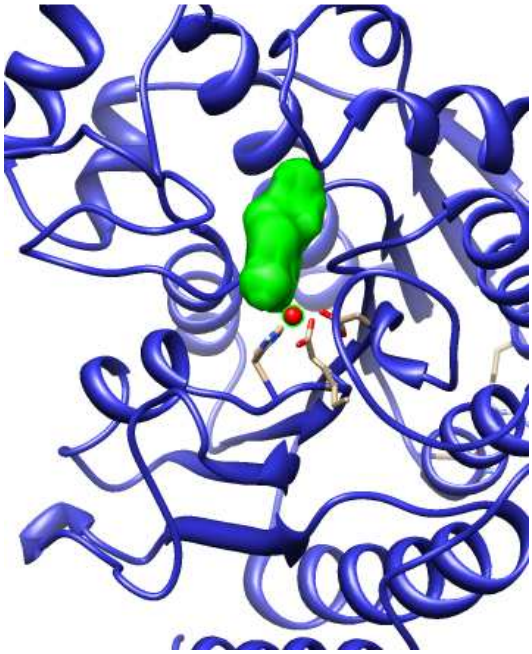


Fig19: HDAC 4 and Allyl disulfide

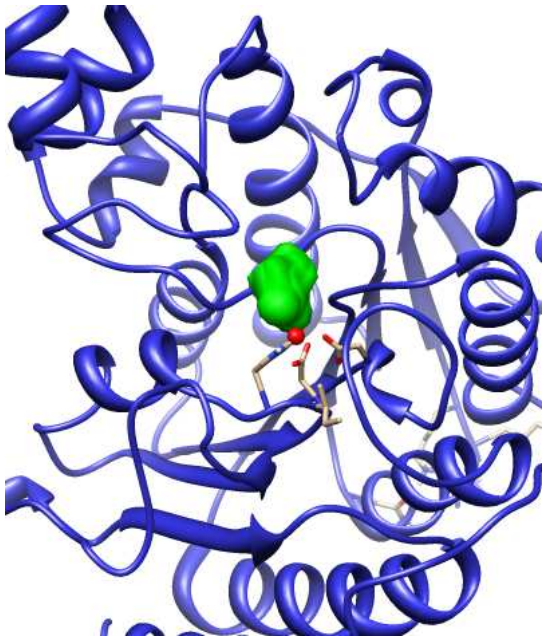


Fig20: HDAC 4 and Butyrate

Figs 17-20: Rrepresents the interactions between inhibitors (sulforaphane, Allyl disulfide, butyrate) in green color and HDAC (4, 6) in light blue color

5.2 Binding Free energy

The result of the docking are save in log format file. These log files contain the binding energy between ligand and protein. The docking result shows that sulforaphane have lowest binding energy in case of HDAC4 in comparison to allyl disulfide and butyrate.

Table2**: The binding free energy docking simulation result of Sulforaphane, Butyrate, Allyl Disulfide toward HDAC(4,6,7)**

	Binding Energy, **Δ G (kcal/mole)**		
Ligands	**HDAC 4**	**HDAC 6**	**HDAC 7**
Sulforaphane	**-177.6**	**-111.9**	**-157.8**
Allyl disulfide	**-157.6**	**-100.6**	**-132.2**
Butyrate	**-134.7**	**-112.4**	**-145.7**

The binding energy of the ligands and protein was plotted in the table 2. The negative binding energy shows that the reactions are spontaneous.

6. Conclusion

From above *in silco* study we can conclude that Sulforaphane shows inhibitory activity towards HDAC (4, 6) as it is interacting with the Zn^{2+} ion of enzyme active site and turn it into inactive form. Whereas allyl disulfide and butyrate only able to interact with active site and Zn^{2+} ion of HDAC4. This experiment is only the *in silico* approach, which need approval after future experiments (*in vivo*). We had tested three inhibitor including, sulforaphane, allyl disulfide and butyrate against HDAC 4,6 and 7. If the future experimental results establish that these three bioactive compounds have potent *in vivo* HDAC inhibition property then it will add a new dimension in cancer treatment.

7. REFERENCES

1. Acharya M, Sparreboom A, Venitz J, Figg W. Rational development of histone deacetylase inhibitors as anticancer agents: a review. Mol Pharmacol 2005;68:917–932. [PubMed: 15955865]
2. Aggarwal B, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 2006; 71:1397–1421. [PubMed: 16563357]
3. Bolden J, Peart M, and Johnstone R Anticancer activities of histone deacetylase inhibitors 2006 Nature Publishing Group 2006 doi:10.1038/nrd2133
4. Catley, L. *et al.,* NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma. Blood 102, 2615–2622 (2003).
5. Choudhuri S, Cui Y, Klaassen C. Molecular targets of epigenetic regulation and effectors of environmental influences. Toxicol Appl Pharmacol 2010; 245:378–393. [PubMed: 20381512]
6. Dalvai M, Bystricky K. The role of histone modifications and variants in regulating gene expression in breast cancer. J Mammary Gland Biol Neoplasia 2010; 15:19–33. [PubMed: 20131086]
7. Davis CD, Ross SA. Dietary components impact histone modifications and cancer risk. Nutr Rev 2007; 65:88–94. [PubMed: 17345961]
8. Ducasse M, Brown M. Epigenetic aberrations and cancer. Mol Cancer 2006;5:60. [PubMed: 17092350]
9. Ellis L, Atadja P, Johnstone R. Epigenetics in cancer: targeting chromatin modifications. Mol Cancer Ther 2009; 8:1409–1420. [PubMed: 19509247]
10. Esteller M. Epigenetics in cancer. N Engl J Med 2008; 358:1148–1159. [PubMed: 18337604]
11. Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., Breslow, R. and Pavletich, N. P. (1999) Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature (London) 401, 188±193
12. Fraga MF, Ballestar, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, *et al.*, Loss of acetylation at Lys 16 and trimethylation at Lys 20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005; 37:391–400. [PubMed: 15765097

13. Ganesan A, Nolan L, Crabb SJ, Packham G. Epigenetic therapy: histone acetylation, DNA methylation and anti-cancer drug discovery. Curr Cancer Drug Targets 2009;9:963–981. [PubMed: 20025605]
14. Görisch S, Wachsmuth M, Tóth K, Lichter P, Rippe K. Histone acetylation increases chromatin accessibility. J Cell Sci 2005; 118:5825–5834. [PubMed: 16317046]
15. Herceg Z. Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. Mutagenesis 2007; 22:91–103. [PubMed: 17284773]
16. Issa JP. Cancer prevention: epigenetics steps up to the plate. Cancer Prev Res (Phila Pa) 2008;1:219–222.
17. J. Xu, J.-Y. Zhou, W.-Z. Wei, S. Philipsen, and G. S. Wu, "Sp1-mediated TRAIL induction in chemosensitization," *Cancer Research*, vol. 68, no. 16, pp. 6718–6726, 2008. View at Publisher · View at Google Scholar · View at PubMed
18. Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, Majid S, Igawa M, Dahiya R. Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells. Int J Cancer 2008; 123:552–560. [PubMed: 18431742]
19. Kim D, Kim M, Kwon H. Histone deacetylase in carcinogenesis and its inhibitors as anti-cancer agents. J Biochem Mol Biol 2003; 36:110–119. [PubMed: 12542981]
20. Kouzarides T. Chromatin modifications and their function. Cell 2007; 128:693–705. [PubMed: 17320507]
21. Lafon-Hughes L, Di Tomaso M, Méndez-Acuña L, Martínez-López W. Chromatin-remodelling mechanisms in cancer. Mutat Res 2008; 658:191–214. [PubMed: 18403253]
22. Landis-Piwowar KR, Huo C, Chen D, Milacic V, Shi G, Chan TH, Dou QP. A novel prodrug of the green tea polyphenol (-)-epigallocatechin-3-gallate as a potential anticancer agent. Cancer Res2007; 67:4303–4310. [PubMed: 17483343]
23. Li Y, Tollefsbol T. Impact on DNA methylation in cancer prevention and therapy by bioactive dietary components. Curr Med Chem 2010; 17:2141–2151. [PubMed: 20423306]
24. M.F. Fraga, E. Ballestar, A. Villar-Garea, M. Boix-Chornet, J. Espada, G. Schotta, T. Bonaldi, C. Haydon, S. Ropero, K. Petrie, N.G. Iyer, A. Perez-Rosado, E. Calvo, J.A. Lopez, A. Cano, M.J. Calasanz, D. Colomer, M.A. Piris, N. Ahn, A. Imhof, C. Caldas, T. Jenuwein,

M. Esteller Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer Nat Genet 2005;37:391–400. [PubMed: 15765097]

25. Majid S, Kikuno N, Nelles J, Noonan E, Tanaka Y, Kawamoto K, Hirata H, Li L, Zhao H, Okino S, Place R, Pookot D, Dahiya R. Genistein induces the p21WAF1/CIP1 and p16INK4a tumor suppressor genes in prostate cancer cells by epigenetic mechanisms involving active chromatin modification. Cancer Res 2008; 68:2736–2744. [PubMed: 18413741]
26. Marks PA. Discovery and development of SAHA as an anticancer agent. Oncogene 2007; 26:1351–6. [PubMed: 17322921]
27. Marks, P. A. & Jiang, X. Histone deacetylase inhibitors in programmed cell death and cancer therapy. Cell Cycle 4, 549–551 (2005). 10. Lindemann, R. K., Gabrielli, B. & Johnstone, R. W.Histone-deacetylase inhibitors for the treatment of cancer. Cell Cycle 3, 779–788 (2004).
28. Meeran S, Ahmed A, Tollefsbol T, Epigenetic targets of bioactive dietary components for cancer prevention and therapy. Clin Epigenetics. 2010 December 1; 1(3-4); 101-116. Doi: 10.1007/s13148-010-0011-5.
29. Meeran S, Katiyar S. Cell cycle control as a basis for cancer chemoprevention through dietary agents. Front Biosci 2008; 13:2191–2202. [PubMed: 17981702]
30. Nebbioso A, Clarke N, Voltz E, Germain E, Ambrosino C, Bontempo P, *et al.*, Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. Nat Med 2005;11:77–84. [PubMed: 15619633]
31. Paluszczak J, Krajka-Kuzniak V, Baer-Dubowska W. The effect of dietary polyphenols on the epigenetic regulation of gene expression in MCF7 breast cancer cells. Toxicol Lett 2010;192:119–125. [PubMed: 19840838]
32. Patra, S.K., Patra, A. and Dahiya, R., (2001). Role of histone deacetylase and DNA methyltransferase in human prostate cancer. Science Direct, 278,705-713.
33. R. R. Rosato, J. A. Almenara, Y. Dai, and S. Grant, "Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induces mitochondrial damage and apoptosis in human leukemia cells," *Molecular Cancer Therapeutics*, vol. 2, no. 12, pp. 1273–1284, 2003.

34. Roth, S. Y., Denu, J. M. & Allis, C. D. Histone acetyltransferases. Annu. Rev. Biochem. 70, 81–120 (2001).
35. S. Fulda, "Modulation of TRAIL-induced apoptosis by HDAC inhibitors," *Current Cancer Drug Targets*, vol. 8, no. 2, pp. 132–140, 2008. View at Publisher · View at Google Scholar
36. Seligson D, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdistani S. Global histone modification patterns predict risk of prostate cancer recurrence. Nature 2005;435:1262–1266. [PubMed:15988529]
37. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010;31:27–36. [PubMed: 19752007]
38. Strahl, B. D. and Allis, C. D. (2000) The language of covalent histone modi®cations. Nature (London)403, 41±45
39. Suter M, Aagaard-Tillery K. Environmental influences on epigenetic profiles. Semin Reprod Med 2009;27:380–390. [PubMed: 19711248]
40. Thiagalingam, S. *et al.,* Histone deacetylases: unique players in shaping the epigenetic histone code. Ann. NY Acad. Sci. 983, 84–100 (2003)
41. Ungerstedt JS, Sowa Y, Xu WS, Shao Y, Dokmanovic M, Perez G, *et al.,* Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. Proc Natl Acad Sci USA 2005; 102:673–8. [PubMed: 15637150]
42. Yang C, Landau J, Huang M, Newmark H. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr 2001;21:381–406. [PubMed: 11375442]
43. Yoshida, M., Kijima, M., Akita, M. and Beppu, T. (1990) Potent and speci®c inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J. Biol. Chem. 265, 17174±17179
44. Yoshida, M., Kijima, M., Akita, M. and Beppu, T. (1990) Potent and speci®c inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J. Biol. Chem.265, 17174±17179
45. Young-Joon Surh 2003; cancer chemoprevention with dietary phytochemicals College of Pharmacy,Seoul National University, Shinlim-dong, Kwanak-ku, Seoul 151-742, South Korea doi:10.1038/nrc1189 Nature reviews (www.nature.com/reviews/cancer)